

# The irreducibly complex ribosome is a unique creation in the three domains of life

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The evolution of the genetic code and the ribosome are intimately connected as the code is expressed through ribosomal activity. Models of genetic code evolution are analyzed. The error-minimization theory is faulty in that it supposes that highly error-prone genetic codes could produce more precise codes over time. The stereochemical theory posits complementarity between nucleotides and amino acids, but cannot demonstrate this for the whole code. The co-evolution theory states that the genetic code developed from an ancestral through an ancient to a modern state. There is no evidence for ancestral code protein generation. The big question remains why the code solidified in its present state. Finally, the accretion theory of ribosomal evolution is shown incapable of answering key questions.

Large and small subunit ribosome proteins are conserved within Archaea, Bacteria, and Eukarya but not between these domains. The size and weight of the subunit proteins are similar between Archaea and Bacteria only, whereas protein types are similar only between Archaea and Eukarya. This implies that the ribosome of all three domains has been created uniquely. The presence of many unique proteins and protein domains in the mitochondrial and chloroplast ribosomal proteins imply that they are not related to prokaryotic ones.

Protein synthesis is a fundamental function in the cell that involves ribosomes. The process involves first the transcription of the DNA sequence to messenger RNA (mRNA). Each sequence of three bases in mRNA is known as a codon. The information contained in the codon is used to produce functional proteins, as each codon specifies a particular amino acid. The vital step in protein formation occurs on the ribosomes with the cooperation of transfer RNA (tRNA) using ribosomal RNA (rRNA) as a binding site. It is evident that ribosomes are prerequisites to the life of the cell in that they convert genetic information into functional proteins. These structures consist of two different sized subunits, whose size is described in terms of Svedberg units (S), which is a measure of the sedimentation rate. Ribosomal proteins dominate these subunit structures, but there are up to 120 different molecules involved: rRNA, mRNAs, tRNAs, ribosomal proteins, aminoacyl-synthetases, and scanning factors. They are all needed to fulfil this basic, yet highly complex cellular housekeeping function. Besides histones (DNA packaging proteins in eukaryotes), ribosomal proteins are the most conserved proteins in the living world.<sup>1</sup>

The coupling of the protein-translation machinery to the DNA is fulfilled in the genetic code, which consists of four nucleotide bases arranged in groups of three (codon). The codons can be arranged in 64 combinations allowing selection of the 20 amino acids; special codons mark the start and stop point for a protein. Some evolutionists believe that the ribosomes came into being before cellular life and represent the first self-replicating entities. This means the ribosomal RNA they carry is a primitive genome.<sup>2</sup>

Here we will take a look at one of the greatest problems in biology. Classically, living systems produce copies of themselves. In ancestral systems, evolutionists consider that the descendants were different from their immediate ancestor as they needed to generate coding rules that then went on to evolve new systems.<sup>3,4</sup>

## Evolutionary theories on the genetic code and the origin of the ribosome

There are up to 22 small ribosomal subunits in *Escherichia coli*, and up to 35 large subunit (LSU) proteins,<sup>5</sup> whereas there are 33 small subunit (SSU) proteins in the human ribosome, compared to 47–48 proteins in the LSU. Across the eukaryotes, the number of ribosome proteins may not be constant.<sup>6</sup> An added complication is that 70S ribosomes are found associated with the plastids (some eukaryotes). Whereas there are similarities in the 70S ribosomes between prokaryotes and eukaryotes, in the machinery associated with translation there are fundamental differences.<sup>7</sup> Other ribosome types have been found in the mitochondria that show many differences in contrast to bacterial ribosomes.<sup>8</sup>

Our focus is on the evolution of the genetic code and the classical 70S prokaryote and 80S eukaryote ribosomes. It is a paradox of evolution that the composition of the prokaryotic ribosome is different to that of the eukaryotic one, yet the ribosome has supposedly evolved through a number of intermediary steps *back into a ribosome*. Evolution simply loses all meaning if a protein or set of proteins evolves into

a structure, which fulfils the same purpose, which it started out from.

Today there are three major theories on the origin of the genetic code:<sup>4,9</sup> the error-minimalization theory,<sup>10</sup> the stereochemical theory,<sup>11</sup> and the co-evolution theory.<sup>12</sup> The recently enunciated accretion theory of ribosomal evolution assumes that the genetic code is also evolving since it supposedly accounts for rRNA, mRNA as well as tRNA changes.<sup>13</sup> Other theories have been proposed,<sup>4,14,15</sup> but these are not discussed except for the accretion model.

The error-minimization or adaptation theory

According to Sonneborn's argument reviewed by Carl Woese,<sup>10</sup> selection pressure acted on a primitive genetic code that led to the generation of a mature genetic code where mutations in codons produced few adverse outcomes in terms of functional proteins. This represents an error-minimization strategy. Woese admitted that the error-minimization scheme involved innumerable "trials and errors" so that it, in his opinion, "could never have evolved in this way".

Others have defended the theory. Some ingenious ideas were admitted subsequently as "utterly wrong". Interestingly, one investigation of the theoretical susceptibility of a million randomly generated codes to errors, through mutations, showed that the standard genetic code was among the least prone to error.<sup>16</sup> This indicated that, if the initial genetic code was primitive and error prone, then what is observed in nature is the best option. However, the question remains as to why only one single code survived. Why not several different ones? This rather stands as evidence that the Creator made a wise choice.

The ancestral translational machinery conceived in evolutionary schemes is, of necessity, very rudimentary, and thus highly prone to errors. This means that it would have been almost impossible to correctly translate any mRNA, and thus produced little more than statistical proteins (proteins with only random sequences). Yet through necessity, somehow, the codons of the ancestral code were gradually reconfigured in order to minimize translational error.<sup>4</sup> The 'somehow' has been imagined as perhaps involving novel amino acids, existence of a positive feedback mechanism that would assign codons to amino acids with similar properties, direct templating between nucleic and amino acids, or other possibilities.<sup>16</sup>

Vetsigian and Woese<sup>17</sup> subsequently proposed that horizontal gene transfer (HGT) could possibly spread workable genetic workable codes across organisms, accounting for the near universality of the genetic code. However, HGT requires that the genetic codes of the host and the recipient species be similar enough for the new genetic code to work. There also needs to be evidence for

a mechanism permitting transfer of genetic information in the ancient past.

The theory naturally cannot carry much weight, since if the translation machinery is so error-prone to begin with, no meaningful proteins can come from such a configuration. Errors only lead to more errors, not higher precision, which requires intelligent input. From a thermodynamic viewpoint, disorder only increases as mutations accrue.

The stereochemical theory

Over the past 60 years, several theories have been set forward which attempt to explain how information in the DNA translates to protein sequences. These are based on some sort of selective stereochemical complementarity or affinity between amino acids and nucleotides (base pair triplets). On a physico-chemical level, this is based on the negative charges of the nucleotide phosphates interacting with the positive charge of the basic amino acids. In Saxinger *et al.*'s study no conclusive selective binding occurred between certain amino acids and nucleotide triplets.<sup>18</sup> More recently, Yarus *et al.*<sup>19</sup> contended that coding triplets arose as essential parts of RNA-like amino acid-binding sites, but they could show this for only seven of the 20 (35%) canonical amino acids. However, they conceded that the code can change.

The take home implication is that different amino acids can be bound by different coding triplets, meaning that the code is not specific and thus meaningless historically. Overall, after decades of research, no evidence has been found which gives strong support to the stereochemical theory. Yarus's group<sup>19</sup> went on to argue that adaptation, stereochemical features and co-evolutionary changes were compatible and perhaps necessary in order to account for present codon characteristics. However, Barbieri<sup>3</sup> has argued that there is "no real evidence in favour" of the stereochemical theory. This serves to illustrate the uncertainty prevailing.

The co-evolution theory

According to the co-evolution theory, the original genetic code was "excessively degenerate" meaning it could code for several amino acids. These originals were used in "inventive biosynthetic processes" to synthesize the other amino acids. The code then adapted to accommodate these new amino acids.<sup>20</sup> Similarities in the codons of related amino acids were subject to computer analysis in order to determine if a better code could be found based on biosynthetically related amino acids. An extraordinary correlation was noted for the universal code, as against 32,000 randomly generated possibilities. Changing the pattern of relatedness among amino acids gave more codes equal to or of greater correlations than the universal code. However, the authors

stated that these observations “cannot be used as proof for the biosynthetic theory of the genetic code”.<sup>21</sup>

Less than half of the 20 canonical amino acids found in proteins can be synthesized from inorganic molecules.<sup>3</sup> Furthermore, the amino acids that are missing (the so-called secondary amino acids) are also missing from material recovered from meteorites.<sup>22</sup> This is problematic for evolution, for it implies that early life-forms on this planet could only use ten amino acids for protein construction, something which we don’t observe today, thereby greatly reducing the possible number of functional proteins.

The primary amino acids were coded by an ancestral genetic code, which then expanded to include all 20 canonical amino acids. The present code is a non-random structure yet it is more robust as far as translational errors are concerned than the majority of alternative codes that can be generated conceptually according to accepted evolutionary trajectories. When the starting assumptions are altered so that the postulated codes start from an advantaged position, then higher levels of robustness are achieved. A better code could have been produced if evolution had continued, but it did not as the possibility of severe adverse effects was too great.<sup>23</sup> This process can be seen in figure 1.

Several questions present themselves here, however. Why don’t we find any protein sequences in the fossils of ancient organisms, which only have primary amino acids? The fact that no such proteins exist is strong proof against the evolutionary origin of the genetic code. We only find proteins made up of all 20 amino acids. Why didn’t the genetic code keep on expanding to cover more than 20 amino acids? Why not 39, 48 or 62? Why did codon triplets evolve, and why not quadruplets? With  $4^4 = 256$  possible codon quadruplets, coding space could have increased, and thus a much larger universe of possible proteins could have been made possible.

An additional fundamental issue is that if life commenced in an RNA world, then amino acids could have been synthesized on the primitive codons associated with these

molecules by primordial synthetases. How do similar coding rules now apply when codon recognition is performed by the anticodons of the tRNA with the assistance of the highly specific aminoacyl-tRNA-synthetases that attach to the amino acids? It has been suggested that perhaps there was a two-base code rather than a three-base one on account of the supposed limited number of amino acids available.<sup>4,24</sup>

The accretion model of ribosomal evolution

The accretion model of ribosomal evolution<sup>13,25</sup> is one of the most recent models and describes how the ribosome evolves from simple RNA and protein elements into an organelle complex in six major phases through accretion, recursively adding, iterative processes, subsuming and freezing segments of the rRNA. It is argued that the record of changes is held in rRNA secondary and three-dimensional structures. Patterns observed in extant rRNA found among organisms were used to generate rules supposedly governing the changes.

First, it is assumed that evolution occurred with changes moving from prokaryotes leading finally to the eukaryotes and with the apex reached with humans. Using this framework, a chronological sequence was constructed of rRNA segment additions to the core structure found in *Escherichia coli*. The six-phase process envisaged provided no evidence for the emergence of ancestral RNA. The proto-mRNA is seen simply as arising from a random population of appropriate molecules. This proto-mRNA together with tRNA, formed through condensation of a cysteine: cysteine: alanine (CCA) sequence unit, gave rise to base-pair coding triplets (codons). The ribosomal units (small and large) are considered to have arisen from loops of the rRNA. The proposed RNA loops were ‘defect-laden’, which required a protection mechanism. During phase 2 the large ribosomal unit is thought of as a crude ribozyme almost as soon as it was a recognizable structure, catalyzing nonspecific, non-coded condensation of amino acids. Finally, the two developing ribosome units came together (phase 4) to form

a complex structure recognizable as a ribosome. In the next phase (5), specific interactions began to occur between anticodons in tRNA and mRNA codons to produce functional proteins. In the final phase the genetic code was optimized.<sup>13</sup>

This narrative suffers from major flaws, some of which also are inherent in previous models of the genetic code generation. No organisms have been found that contain ribosomes in any of these intermediary phases. If these intermediary phases are capable of

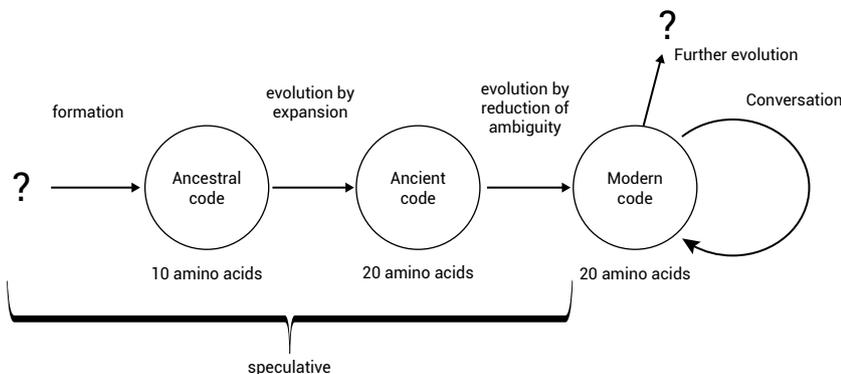


Figure 1. Different steps in the evolution of the genetic code according to the co-evolution theory

ribosomal function, then why was it necessary to evolve further during additional steps? An insistent problem is how a genetic code could be generated that depends for its expression on proteins that can only be formed when it exists. Petrov *et al.*<sup>25</sup> proposed a partial solution. The peptidyl transferase (enzyme) centre, an essential component of the ribosome, arose from an rRNA fragment. This means that its origin is conceived of as being in the RNA world.<sup>26</sup> The peptidyl transferase centre is the place in the 50S LSU where peptide bond synthesis occurs. The machinery is very complex in extant organisms. In its original incarnation, the embryonic centre was less than 100 nucleotides long. The original RNA world quickly morphed into the familiar RNA/protein world. This argument is necessary as it “has proven experimentally difficult to achieve” a self-replicating RNA system. In a revealing aside, Fox even suggested that perhaps it is not necessary to validate the existence of the RNA world if it had a short life.<sup>26</sup>

Some of the additional problems with an RNA world origin were noted by Strobel.<sup>27</sup> An RNA commencement to life on Earth rests on the ability of RNA to both share the task of encoding and also to replicate information. This proposition depends on the abilities of RNA copying enzymes (ribozymes). However, such enzymes are unable to copy long templates and at a sufficient rate to overtake decomposition processes. Even greater issues are that there is no sensible resolution to the question of the origin of the activated nucleotides through abiotic processes needed for RNA formation, or of the problem as to how randomly assembled nucleotides achieved the ability to replicate. This has led some to conclude that “the model does not appear to be very plausible”. Nevertheless, undaunted, other possibilities have been invented.<sup>28</sup>

### The irreducibly complex character and conservation of the ribosome

The foregoing discussion leads us to conclude that the ribosome in itself is an irreducibly complex cellular organelle, requiring several dozen proteins to be present at the same time in order for it to work. Furthermore, the molecular machinery that regulates ribosomal gene co-expression involves just under 300 transcription regulators, which is also further modulated according to several cell types in humans and mice.<sup>29</sup> The ribosome is also a prime example of the evolutionary paradox of sequence conservation of both functional genes and regulatory sequences.<sup>30</sup> Among the approximately 60 proteins that are represented by an ortholog (gene in different species retaining the same function) in every single cellular life-form with a sequenced genome, over 50 are components of the translation machinery.<sup>31</sup> Some of these ribosomal protein genes (RPGs) are necessary for function or required for

self-assembly, whereas others can be used for stimulation of the translation process.<sup>4</sup> Ribosomal dysfunction is in the background of at least a dozen diseases, including some forms of cancer (table 1) (see appendix<sup>62</sup>).<sup>32</sup> This is evidence that these proteins could not have evolved gradually over time, but are part of a complex functional unit.

Evolutionists argue that during ribosomal evolution, different proteins were co-opted from other processes. The ribosome is made up of approximately 80 proteins, around half of which play an exclusive role within the ribosome. The remainder of those studied have extra-ribosomal functions (although seven of them have not yet been studied, according to Wang *et al.*).<sup>33</sup> Thus, since half of all ribosomal proteins (RPs) have no role outside of the ribosome, the co-option argument does not appear realistic.

The conservation of the 80 ribosome RPs can be studied across a range of species using the Ribosomal Protein Gene Database (RPGD).<sup>34</sup> For each protein, the number of database sequences, the number of species, and the percent conservation were noted (see materials and methods section). We found that 48.8% (39 out of 80) RPs from the database had at least 50% sequence conservation (table 2) (see appendix<sup>62</sup>). In a study of 41 of the 54 RPs from *Escherichia coli*, these genes were individually deleted to verify whether they are necessary for ribosomal function. Of these 41 proteins, nine (22%) were shown to be non-essential (RPs S9, S17, L15, L21, L24, L27, L29, L30, and L34).<sup>35</sup> This is taken to mean that the ribosome performs basic functions essential for existence and that the operational design is robust hence accounting for the use of common elements. Further, the RPGD (136 RP alleles) was compared to the *Saccharomyces* Genome Database,<sup>36</sup> to see what effect the null mutation (when the gene is functionally knocked out) had on each individual gene. It was found that 113 alleles showed a reduced phenotype, 22 were unviable, and one had no information. This was again taken to indicate the functional necessity of the majority of alleles.

Related analyses have been extended to prokaryotes. Yutin *et al.*<sup>37</sup> counted the number and distribution of RPs in a study of 995 bacterial species and 87 archaeal species. The level of conservation of the proteins is shown in figures 2 and 3. Fifty six out of 71 (78.9%) RPs were conserved in all 87 archaeal species, and 44 out of 56 (78.6%) RPs are conserved in all 995 bacterial species. Of the small and large subunit proteins, which are not universally conserved among the 995 bacterial species, S21 and L17 are found on the surface of the ribosome in *E. coli*, suggesting that they are free to mutate, and are thus unessential. Of these two, S21 was deleted from *E. coli* via  $\lambda$ -Red-mediated recombination.<sup>35</sup> Only four RPs that are not present in the 995 species are not exposed on the surface of *E. coli*.<sup>38</sup> Of these, S16 and L31 were present in 99.9%, L34 in 99.7%, and L30 was present in 85.4% of all



involve the presence of suppressor sequences that permit viability of mutants, and the presence of duplicate genes. Culture conditions may contribute to failures in correct identification, as well as technical problems involving primer design.<sup>40</sup> Apart from these difficulties, a range of ribosomal proteins has been identified that have no apparent vital function. Whereas some of these conclusions may be correct, statements about non-essentiality will undoubtedly be revised as more sophisticated methods are used and a greater range of organisms is analyzed. One caution is merited. When investigators write about the non-essentiality of a gene, which gives rise to non-essential proteins, this does not necessarily mean that the absence of such a gene has no effect. This is illustrated nicely with *Saccharomyces*. The single copy of the RPL29 gene is considered non-essential. It codes for a 60S ribosomal subunit protein (Rpl29p). However, its absence retards the coupling of 60S and 40S subunits and also translation events. There are also interactions between RPL29 and essential genes.<sup>42</sup>

### Taxonomic distribution of ribosomal proteins and patterns of gene loss for ribosomal proteins

Taxonomic distribution of ribosomal proteins between the three domains of life

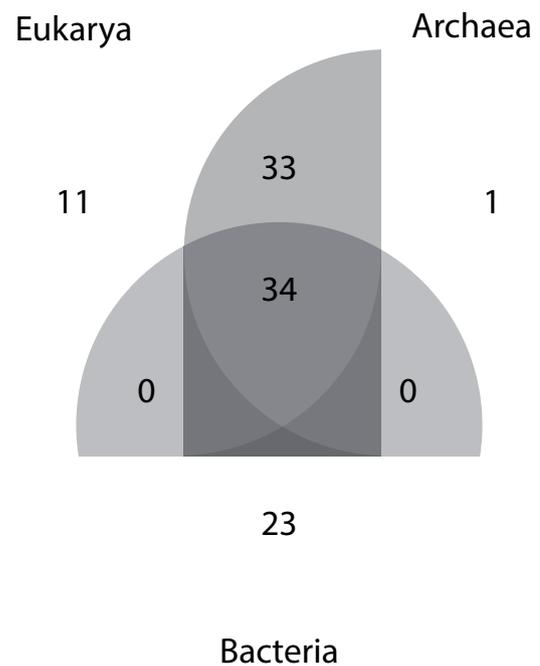
According to Fox<sup>26</sup> and Barbieri<sup>4</sup> the ribosome would have existed in essentially its modern form by the time of the Last Universal Common Ancestor (LUCA). However, there is variation across the three domains of life. Within each domain conservation is stronger than between domains.<sup>43</sup>

**Table 3.** Degree of conservation of proteins in different domains within the small ribosomal unit (SSU) and the large ribosomal unit (LSU)

| Group  | SSU           | LSU           | Reference              |
|--|---------------|---------------|------------------------|
| Archaea  | 18/22 (81.8%) | 26/34 (76.5%) | Yutin <sup>37</sup>    |
| Bacteria   | 24/28 (85.7%) | 32/43 (74.4%) | Yutin <sup>37</sup>    |
| Archaea, Bacteria, Eukarya                             | 15/24 (62.5%) | 20/38 (52.6%) | Mears <sup>39</sup>    |
| Archaea, Bacteria, Eukarya                             | 15/40 (37.5%) | 17/62 (27.4%) | Lecompte <sup>44</sup> |
| Archaea, Bacteria, Eukarya + mitochondria, chloroplast | 13/24 (54.2%) | 9/38 (23.7%)  | Mears <sup>39</sup>    |

**Table 4.** Pearson correlation between Archaea, Bacteria, and Eukarya according to the absence/presence of different RPs in their genome

|         | Archaea + Bacteria | Archaea + Eukarya | Bacteria + Eukarya |
|---------|--------------------|-------------------|--------------------|
| SSU     | 0.32               | 0.7               | 0.03               |
| LSU     | 0.03               | 0.71              | -0.31              |
| SSU+LSU | 0.14               | 0.71              | -0.19              |



**Figure 5.** The number of ribosomal protein genes common and unique to Archaea, Bacteria, and Eukarya

Out of in excess of a hundred ribosomal protein genes (RPGs), 32 were conserved in all three main domains of life (Eukarya, Archaea, and Bacteria), 33 are common to only Eukarya and Archaea, whereas there are no RPGs specific to Eukarya and Bacteria or Archaea and Bacteria. Twenty-three RPGs are unique to Bacteria, whereas 11 are unique to Eukarya, and only 1 to Archaea (figure 5).<sup>44</sup>

Márquez *et al.*<sup>45</sup> also discovered nine RPs which hypothetically might be unique to Archaea. According to the Lecompte data, Archaea+Eukarya have a 0.7–0.71 Pearson correlation coefficient with each other, according to the absence/presence of RPs in the SSU, the LSU, or in both (table 4). The data as a whole poses a recognized problem in that no clear evolutionary scenario emerges and one is left to ponder whether prokaryotes emerged from an ancestral eukaryote genome, a bacterial-like genome or whether symbiosis was involved,<sup>42</sup> as we will elaborate on later.

The information is noteworthy to us too, since eukaryotes have a 60S large subunit (LSU), and a 40S small subunit (SSU), whereas prokaryotes such as Archaea and Bacteria have a 50S LSU and a 30S SSU. This would imply that the ribosomes of Archaea and Bacteria are structurally different from than that of Eukaryotes. However, the absence/

presence correlation of RPs discussed previously suggests that the ribosomes of Archaea and Eukarya also group away from Bacteria. This suggests to us that Archaea, Bacteria, and Eukarya are all distinctly created domains of life.

Ribosomal proteins of the mitochondrion and chloroplast

Eukaryotic mitochondrial RPs have originated from prokaryotes in the view of some evolutionists. They point as evidence to size similarity between the two types of ribosomes, as well as sequence homology. However, mitochondrial ribosomes are missing some RPs present in prokaryotes, and have unique proteins of their own, which are not homologous to bacterial RPs.<sup>46</sup> These unique proteins replace the function of rRNAs in the prokaryotic organisms, such as the 50% of rRNAs that correspond to a RP in the protist *Leishmania tarentolae*.<sup>47</sup> When ribosomal proteins of *E. coli* and the human mitochondrial ribosome are compared (table 5) (see appendix<sup>62</sup>), only 42 of the 88 proteins (47.7%) are common between the two types of ribosomes, with 12 being unique to *E. coli*, and 34 unique to the human mitoribosome. In a further example, yeast MRP51 gene encodes a novel protein responsible for global mitochondrial translation.<sup>48</sup> Again, the function of MPS33 in *Drosophila melanogaster* is unknown, but its absence can cause cardiomyopathy<sup>49</sup> and MRPL55 has been shown to be necessary for mitochondrial biogenesis.<sup>50</sup>

Similar to the mitochondrion, the chloroplast ribosome is also missing RPs compared to the prokaryotic ribosome, and also contains RPs not found in the ribosomes of prokaryotes. The RP content of *E. coli* and *Chlamydomonas reinhardtii* is compared in table 5. There is an 80.3% commonality.<sup>51</sup> Despite the high number of common RPs, the chloroplast RPs that are in common with Bacteria also have novel protein domains. For example, a plastid-specific ribosomal protein (PSRP-3) takes part in the regulation of translation, whereas PSRP-7 assists the positioning of mRNA during translation initiation. RAP38 and RAP41 are ribosome-associated proteins, which take part in translation.<sup>52</sup>

Once again, just because prokaryotic ribosomes are superficially similar to eukaryotic mitochondrial ones, does not necessarily mean that they are descended from them.<sup>53</sup>

Loss of ribosomal protein genes (RPGs)

Evolutionists surmise that, with the exception of the LXa gene, the full complement of RPs was present in the ancestor of Archaea and Eukarya, with reductive evolution happening on the scale of a whole domain. Furthermore, this led Lecompte *et al.*<sup>44</sup> to suggest that the full complement of archaeal RPs was present in the cenancestor of both Archaea and Eukarya, leading to the conclusion that the prokaryotes evolved by simplification of this ancestral eukaryotic-like genome, an idea suggested by several

authors.<sup>54,55</sup> This creates an uncomfortable dilemma for evolutionists in that they have to choose between such a theory of origin as opposed to one based on a symbiotic hypothesis involving eubacteria and methanogenic Archaea or even other possibilities.<sup>56</sup> An added dilemma is introduced in that it is admitted that closeness of sequence similarity need not mean a close phylogenetic relationship unless there were similar rates of evolution involved with the different lineages considered.<sup>57</sup>

RPG losses are restricted to small numbers of divergent species or genera, meaning that these gene deletion events occur independently in these lineages. RPG losses tend to happen more frequently in intracellular pathogens, such as *Mycoplasma genitalium*, *M. pneumoniae* and *Encephalitozoon cuniculi*.

The LSU RNA of the microsporidian *Encephalitozoon cuniculi* is greatly reduced in length compared to other eukaryotes, and so is its SSU RNA, which is only ~1300 bp, as compared to ~1600 bp in prokaryotes and ~1800 bp in other eukaryotes. Furthermore, the internal transcribed spacer DNA 2 region (ITS2) located between the 5.8S and 28S region in eukaryotes is lacking in *E. cuniculi*, suggesting, to those seeking evolutionary clues to origins, that this species is one of the earliest eukaryotic species to diverge from prokaryotes.<sup>58</sup> However, the parasitic lifestyle of this species suggests otherwise, namely that the loss of the ITS2 spacer and the smaller size of the two ribosomal RNA subunits indicates that it has been derived from other eukaryotes and not prokaryotes. Further analysis of *E. cuniculi* has indicated that it contains mitochondrial genes (e.g. the Fe-S cluster assembly), suggesting that this organism group arose as a result of degenerative processes.<sup>59</sup> The clear implication is that though some prokaryotes may show similarities to eukaryotes this doesn't mean that such features could not have come from prokaryotes.<sup>53</sup> Conversely, certain prokaryotic features present in eukaryotes also do not necessarily mean that eukaryotes evolved from prokaryotes.

## Conclusion

The ribosome is an example of sudden, early complexity, if evolution is true. Its appearance raises many speculative events, sequences, and unresolved issues,<sup>60</sup> making such a scenario highly dubious. A number of theories surrounding the evolution of the genetic code and of ribosomal evolution have been examined, but have been found lacking in any form of convincing evidence. Rather, due to its content and intricacy of operation, the ribosome is an example of irreducible complexity, with several dozen proteins making up its functional-structural core. The ribosome differs in make-up between Archaea, Bacteria, and Eukarya, with a number of RPs which are unique to each, suggesting

that these structures did not evolve from each other but rather came into being separately by special creation. Also, some of the ribosomes in prokaryotes are missing from the mitochondria and chloroplasts of eukaryotes. Novel proteins have been found. This is taken to highlight their independent creation. The existence and distribution of RPs does not make sense, except in the light of creation.

### Materials and methods

Figures 2 and 3 were drawn in Excel. Figure 4 was made using the RiboVision software (Bernier *et al.*<sup>61</sup>). Figure 5 was made using the Venn diagram software at bioinformatics.psb.ugent.be/webtools/Venn/. The multiple alignment of all 80 eukaryotic RPs were downloaded from the Ribosomal Protein Gene Database at ribosome.med.miyazaki-u.ac.jp/. A perl script was used to calculate the degree of conservation in each of the proteins. A position in the multiple alignment was taken to be conserved if any given amino acid was present in at least 80% of the sequences. Only those positions were counted where at least 90% of the characters were not a gap character (“-”). The data for table 1 was taken from Narla *et al.*<sup>32</sup> and Wang *et al.*<sup>33</sup> Deletion information was taken from the *Saccharomyces* Genome Database<sup>36</sup>. Ribosomal protein annotation for *Chlamydomonas reinhardtii* was taken in part from the Joint Genome Institute (JGI) at genome.jgi.doe.gov/Chlre3/Chlre3.download ftp.html.

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